

DESCRIPTION OF THE FIRST *ROBOASTRA* SPECIES (NUDIBRANCHIA, POLYCERIDAE, NEMBROTHINAE) FROM THE WESTERN ATLANTIC

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ABSTRACT

A new species of polycerid nudibranch of the genus *Roboastrea* (*Roboastrea ricei* sp. nov.) is described from its type locality off Florida; it is also known from North Carolina. The animal is dark yellow with numerous dark blue, almost black, small spots scattered on the notum and both sides of the body. The oral tentacles, the rhinophores, the posterior part of their rhinophore sheaths and the gill branches are dark blue at the tip, light blue in the middle, and yellow at their bases. The edge of the foot is also dark blue. Two other species of the genus, *Roboastrea europaea* García Gómez, 1985, and *Roboastrea caboverdensis* Pola, Cervera and Gosliner, 2003, are known from the Eastern Atlantic Ocean, but have not been found in the Western Atlantic. *Roboastrea ricei* appears as the sister taxa of the two Eastern Atlantic species, although this relationship is not well supported. This description brings the number of species of the genus to seven. The distinctive color pattern, differences in the radula and reproductive system and its geographical distribution characterize this species and clearly distinguish it from all other described species. Similarly, COI and 16S mitochondrial nucleotide sequences clearly distinguish this species as distinct from all other members of the clade. Based on morphological and molecular phylogenetic analyses, *Tambja tentaculata* Pola, Cervera and Gosliner, 2005, fits within the clade of *Roboastrea* species. In order to maintain the monophyly of *Roboastrea*, we transfer this species to *Roboastrea*.

The taxonomy and phylogeny of the genus *Roboastrea* has been reviewed in the last few years (Pola et al., 2003, 2005, 2007). As a result of these studies, two new species were added to this genus, *Roboastrea caboverdensis* Pola, Cervera, and Gosliner, 2003, from the Cape Verde Archipelago and *Roboastrea leonis* Pola, Cervera, and Gosliner, 2005, from the Galápagos Islands, while *Roboastrea rubropapulosa* (Bergh, 1905) was considered as synonym of *Roboastrea gracilis* Bergh (1877) and *Roboastrea arika* Burn, 1967, was considered as *nomen dubium* (Pola et al., 2005). Features shared by members of the genus *Roboastrea* (sensu Burn, 1967) are the well-developed oral tentacles as dorso-laterally grooved cylindrical projections, small salivary glands, the presence of elongated pouches at the junction of the oral tube and the buccal bulb, oral tube long and penis with spines arranged in helicoidal rows. Moreover, the preliminary phylogeny based on morphological analysis (Pola et al., 2005) indicated that the genus *Roboastrea* was monophyletic with high bootstrap support, with *R. gracilis* and *Roboastrea luteolineata* Baba (1936), both from the Indo-Pacific, being the basal species of this clade. Later, Pola et al. (2007) showed that the recently described species, *Tambja tentaculata* Pola, Cervera, and Gosliner, 2005, should be included within *Roboastrea* in order to maintain the monophyly of the genus.

Here we describe the first Western Atlantic species of *Roboastrea*. The coloration of this species is unique among the species of the genus, and the presence of dorso-laterally grooved oral tentacles together with its internal anatomy permits us to include it within the genus *Roboastrea*.

MATERIAL AND METHODS

The material examined is deposited at the California Academy of Sciences, San Francisco (CASIZ). The specimen was dissected by dorsal incision to facilitate a morphological examination. The internal features were examined using a dissecting microscope with a camera lucida. The penis and the oral tube were critical point dried for scanning electron microscopy. The buccal mass was dissolved in 10% sodium hydroxide until the radula was isolated from the surrounding tissue. The radula was then rinsed in water, dried, and mounted for examination by scanning electron microscopy. Features of living animals were recorded from photographs and notes by collectors. Partial sequences of the mitochondrial cytochrome *c* oxidase subunit I (COI) and mitochondrial large ribosomal subunit (16S rRNA) from the holotype were amplified by polymerase chain reaction (PCR) using the following primers: LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') (Folmer et al., 1994) for COI and 16Sar-L (5'-CGC CTG TTT ATC AAA AAC AT-3') and 16Sbr-H (5'-CCG GTC TGA ACT CAG ATC ACG T-3') (Palumbi et al., 1991) for 16S rRNA. (GenBank accession numbers COI: EU735164, 16S: EU735163). We used the same methodology for DNA extraction, amplification, and sequence alignment as used in Pola et al. (2007). Since Nembrothinae was stated to be a monophyletic clade (Pola et al., 2007), we added these new sequences to a sub-dataset of our previous combined molecular dataset. The taxon sampling used in this analysis contained 41 specimens of Nembrothinae plus 4 specimens of Polycerinae and 3 specimens of Triophinae (see Pola et al., 2007). The specimens considered as *Nembrotha* sp. 3 and *Nembrotha* sp. 4 in Pola et al. (2007) are here named *Nembrotha aurea* and *Nembrotha rosannulata*, respectively (Pola et al., 2008).

We performed a Bayesian analysis to estimate the posterior probabilities of the nodes in the phylogenetic tree. Mr Bayes 3.0b3 (Huelsenbeck and Ronquist, 2001) was run with 6 substitution types (nst = 6). This procedure is based on a GTR model and considers gamma distributed rate variation as well as the proportion of invariable positions for the two genes combined. Data from each of the two genes were treated as different data partitions. Analyses were initiated with random starting trees and run for 2,000,000 generations. The Markov chains were sampled each 100 generations. Of the resulting trees, 2100 were discarded as "burn in". *Crimora lutea* Baba, 1949 was chosen to root the tree.

SYSTEMATICS

DORIDOIDEA

Family POLYCERIDAE Alder and Hancock, 1845

Subfamily Nembrothinae Burn, 1967

Genus *Roboastra* Bergh, 1877

Type species: Roboastra gracilis Bergh, 1877: 458, pl. 56, figs 11–17

***Roboastra ricei* new species**

(Figs. 1–3)

Tambja cf. *gratiosa* (Bergh, 1890): Rudman, 2005 (July 19)

Roboastra cf. *gratiosa* (Bergh, 1890): Valdés et al., 2006 : p. 140

Tambja gratiosa (Bergh, 1890): Debelius and Kuitert, 2007: p. 77

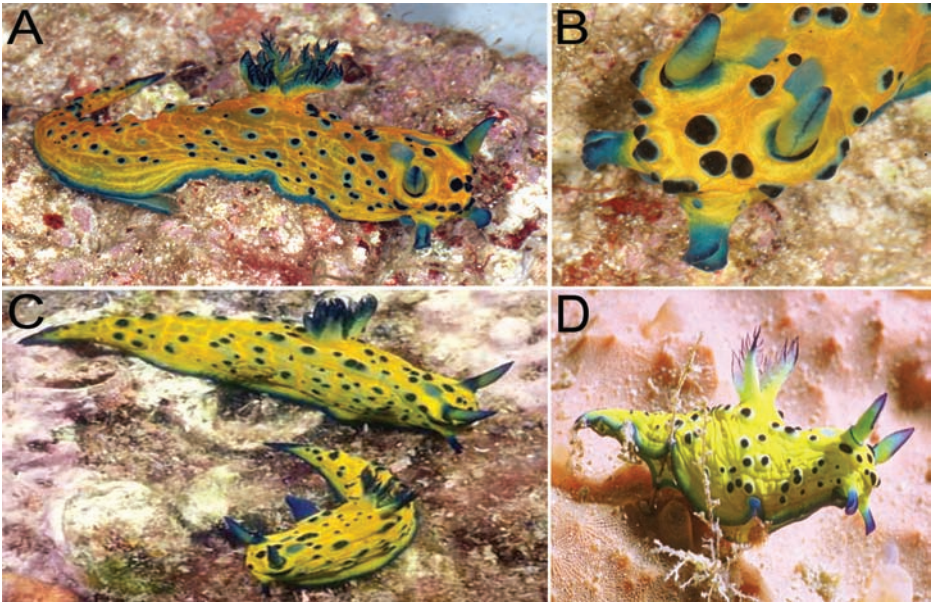


Figure 1. (A–B) *Roboastrea ricei* n. sp., living animal (holotype, CASIZ 173900), Florida, 5 mi off shore of Loran Tower, halfway between the St. Lucie Inlet in Stuart and Jupiter Inlet. (27°03.845 N, 80°02.069 W), 2006, 23 m depth, photo by T. M. Gosliner. (C) *R. ricei* n. sp., living animal, Moorehead City, North Carolina, Atlantic Ocean, 17 m depth, length: 51 mm, 1992, photo by Herb Segars. (D) *R. ricei* n. sp., living animal, northwest Florida, Gulf of Mexico, length: 13 mm, photo by Carol Cox.

Type Material.—**Holotype:** CASIZ 173900, 1 specimen, 23 m depth, Florida, 5 miles off shore of Loran Tower, halfway between the St. Lucie Inlet in Stuart and Jupiter Inlet. (27°03.845 N, 80°02.069 W), 04 March 2006. Collected by Bob Rice.

External Morphology (Fig. 1).—The body is elongate and limaciform with a long and pointed posterior end of the foot. The preserved animal is 40 mm long. The body surface of the living animal is strongly wrinkled. The head is rounded with a pair of conical, completely retractile, perfoliate rhinophores with about 45–50 tightly packed lamellae. The eyespots are bluish in color and well defined. The oral tentacles are strongly developed and dorso-laterally grooved along a part of their length (Fig. 1B). There are five non-retractile tripinnate gill branches, all of them similar in length. The gill branches form a semicircle surrounding the anal papilla. The genital pore opens on the right side, midway between the gill and rhinophores. The background color is dark yellow with numerous spots scattered on the notum and both sides of the body. The color of the spots is dark blue, almost black, bordered by greenish blue. The oral tentacles, the rhinophores, the posterior part of their rhinophore sheaths and the gill branches are dark blue at the tip, light blue in the middle and yellow at their basis. The inner and outer branchial rachides follow the same color pattern. The foot is dark blue bordered by greenish blue.

Internal Morphology.—The anterior digestive tract begins with a long thick-walled muscular oral tube, that continues into the buccal mass. At their junction, a pair of elongate pouches open into the digestive system (Fig. 2A). The surface of these structures is covered with many cilia (Fig. 2B). There is a pair of small, short wide salivary glands on the buccal mass, flanking the esophagus. The radular formula is $33 \times 3.1.1.1.3$ (Fig. 2C). The rachidian tooth (Fig. 2C) is broad, clearly arched at its base,

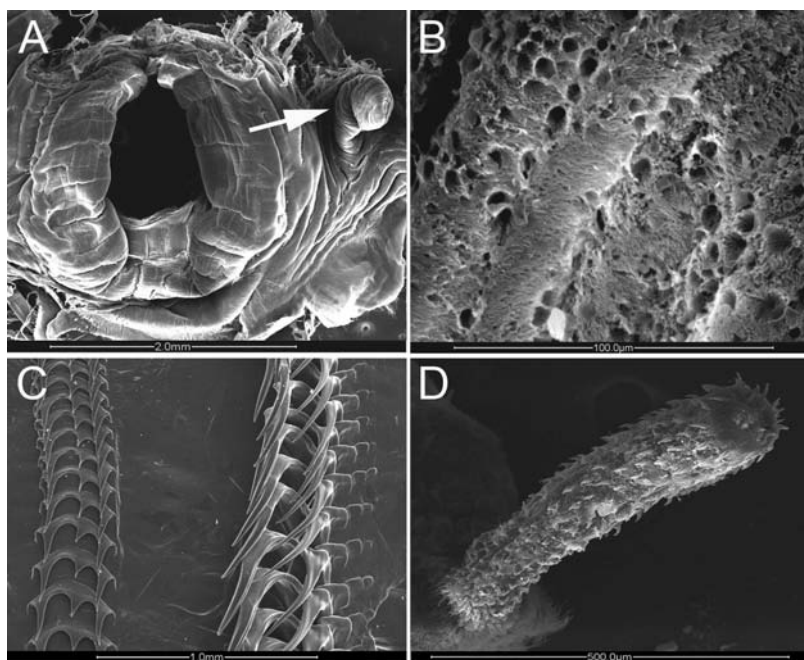


Figure 2. *Roboastra ricei* (CASIZ 173900), scanning electron micrographs. (A) junction of the oral tube and the buccal mass. The arrow shows the presence of an elongate pouch into the digestive system. (B) detail of the surface of right pouch. (C) right half of the radula. (D) penis.

having three well-differentiated cusps; the outer two cusps longer than the middle one. The inner lateral tooth (Fig. 2C) is hooked with two well-developed elongate, sharp cusps. The inner cusp is simple and larger than the outer one, which is very slender. The outer lateral teeth (3) are smaller and quadrangular without prongs, and decreasing in size from the inner to the outer side of the radula. A labial cuticle is present, but lacks armature (Fig. 2A).

Reproductive System.—The reproductive system is triaualic (Fig. 3). The hermaphroditic duct widens into a strong S-shaped ampulla. The ampulla narrows into a large and wide postampullary duct, which bifurcates into the vas deferens and oviduct. The short oviduct enters the female gland mass. The deferent duct, which lacks a morphologically well-differentiated prostate, is long and coiled, ending in a dilated penial sac. The deferent duct has a uniform width, but it is slightly wider and thicker in the prostatic part. The penis is located within the distal end of the deferent duct, and it is armed with at least three different kinds of hooked and chitinous spines arranged in helicoidal rows (Fig. 2D). The bursa copulatrix is rounded and the seminal receptacle is elongate, the latter a bit longer than the former. The seminal receptacle joins with the vagina, near the bursa via a long duct. The vagina is long and straight. There is a large, rounded, thick-walled, vaginal gland, which joins the distal part of the vagina; both open into the genital atrium.

Etymology.—This species is named after Bob Rice, who kindly collected the specimen.

Distribution.—Thus far, known from Florida (type locality; Cox, 2005) and North Carolina (Clifford, 2005; Segars, 2005; Debelius and Kuitert, 2007). According to Valdés et al. (2006) this species was found in South Carolina but as a matter of fact the photograph (p. 140) was taken in North Carolina (H. Segars, pers. comm.).

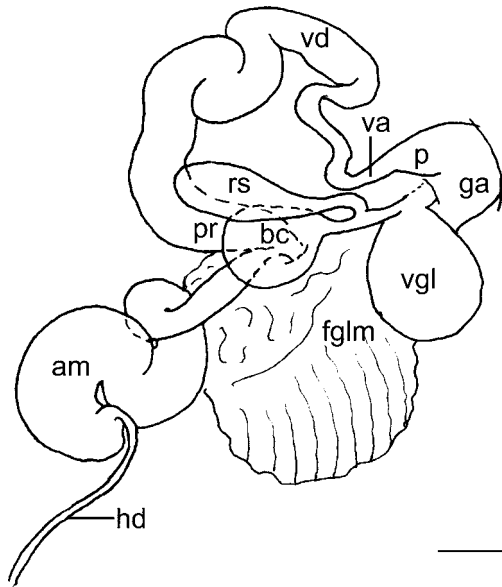


Figure 3. Drawing of the reproductive system of *Roboastra ricei* (CASIZ 173900). Abbreviations: am = ampulla; bc = bursa copulatrix; fglm = female gland mass; ga = genital atrium; p = penis; pr = prostate; rs = receptaculum seminis; va = vagina; vd = vas deferens; vgl = vaginal gland.

DISCUSSION

Roboastra ricei sp. nov. is clearly distinguishable from other species of *Roboastra* previously described, even though only one specimen was available for examination. This species was first noted in a message sent to the Sea Slug Forum (Segars, 2005). Segars had photographed two specimens more than 10 yrs ago off the coast of North Carolina on the shipwreck *CARIBE SEA*. Three days later, another message with a new picture of this unnamed species was sent to the same forum by another photographer (Cox, 2005). The latter specimen was found in northwest Florida and it had well marked white circles around the large blue spots. Clifford (2005) photographed another specimen on the same wreck where Segars took his photo ten years previously, and this was sent to the same website. Thus, the presence of this species had been noted several times but no specimens were available until the specimen examined herein was collected. During these past years, this species has been tentative identified as *Tambja? gratiosa* (Bergh, 1890) (Rudman, 2005), *Roboastra gratiosa* (Bergh, 1890) (Valdés et al., 2006) and *T. gratiosa* (Debelius and Kuiter, 2007) based only on the previous mentioned photographs (Clifford, 2005; Cox, 2005; Segars, 2005; Valdés et al., 2006). The original description of *Nembrotha gratiosa* Bergh, 1890 said that the background color was yellow with blue-black spots, the rhinophores were blue-black above and yellow below, and the gills were yellow below with blue-black edges. The author also described the edge of the foot with a blue-black line. This description matches with the external color pattern of *R. ricei* but Bergh (1890) also described the presence of a caudal crest, similar to that of *Tambja divae* (Marcus, 1958), which

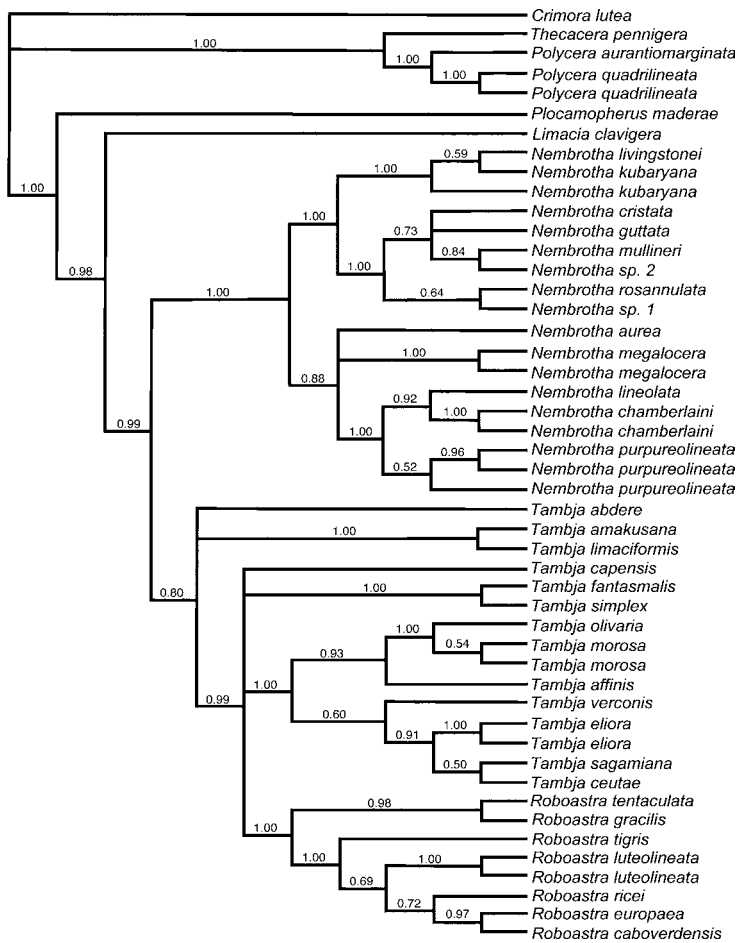


Figure 4. Phylogenetic hypothesis based on combined molecular data (COI+16S) represented by Bayesian inference. Numbers above branches represent posterior probabilities. The name *Nembrotha nigerrima* (in Pola et al., 2007) has been changed to *N. kubaryana* based on Rudman (2008) and Gosliner (2008).

is absent in *R. ricei*. But, more importantly, Bergh (1890) also figured the oral tentacles, the radula, and the penial spines of *N. gratiosa* and those are very different from those described here for *R. ricei*, the former being more similar to a species of the genus *Tambja* Burn, 1962 (Pola et al., 2006).

Externally, *R. ricei* differs from the other *Roboastra* species in the presence of numerous small spots scattered all over the surface of the animal, unique to this species. The majority of Pacific and Atlantic species of *Roboastra* have a pattern of longitudinal lines (see Pola et al., 2005), but this is absent in *R. ricei*. In some specimens of *R. gracilis* the longitudinal lines are broken into a series of discontinuous spots, but these are arranged in a quite specific way. Moreover, the coloration of *R. ricei* is completely different from the remaining species of the genus. While most of the described species of *Roboastra* have yellow coloration on the longitudinal lines, the background color of *R. ricei* is mostly yellow with dark blue or black spots bordered by greenish blue. Anatomically, the inner lateral teeth of *R. ricei* have two very well developed cusps, with the inner cusp being simple, while in *R. gracilis*, *R. luteo-*

lineata, and *Roboastrea europaea* García Gómez, 1985, the inner cusp is bifid. This simple cusp of the inner lateral tooth is also present in *R. caboverdensis*, *R. leonis*, and *Roboastrea tigris* Farmer, 1978, but in the latter two, the central cusp of the rachidian teeth are less developed than in *R. ricei*. *Roboastrea caboverdensis* has the most similar radula to *R. ricei* but in *R. caboverdensis* the outer cusp of the inner lateral teeth is more robust than in *R. ricei*. Regarding the reproductive system, *R. ricei* has a much more rounded vaginal gland and the seminal receptacle is more elongate than in the other species of the genus, but these differences need to be verified in additional material when it becomes available.

When we include *R. ricei* in a sub-dataset of our previous combined molecular data including all available Nembrothinae samples plus some specimens of Polycerinae and Triophinae (Pola et al., 2007), *R. ricei* fits within the well-supported clade (pp = 1.00) containing all *Roboastrea* species (Fig. 4). *Roboastrea gracilis* and *T. tentaculata* are sister taxa and basal to the very-well supported clade (pp = 1.00) containing all remaining *Roboastrea* species. Within this latter clade, *R. ricei* is sister taxa to the clade including the two other Atlantic species, *R. europaea* and *R. caboverdensis*, although this relationship is not strongly supported (pp = 0.72). Divergences in the COI gene between *R. ricei* and its closest relatives are 7.29% (*R. ricei* vs *R. europaea*) and 7.45% (*R. ricei* vs *R. caboverdensis*), with even greater divergences with other members of the clade.

We assign *R. ricei* to the genus *Roboastrea* in accordance with the diagnoses by Burn (1967) and Pola et al. (2005) who defined this clade as having oral tentacles developed as dorso-laterally grooved cylindrical projections, small salivary glands, presence of well developed elongated pouches at the junction of the oral tube and the buccal bulb, oral tube long, rachidian teeth with three well developed denticles, prostate gland small, confined to a coiled glandular section of the vas deferens, and penis with three different types of spines arranged in helicoidal rows. Otherwise, *T. tentaculata* shares only one morphological characteristic with the members of *Roboastrea*, the presence of oral tentacles developed as dorso-laterally grooved cylindrical projections. However, despite the taxonomical consideration of the genera *Roboastrea* and *Tambja* given by Pola et al. (2007), that morphological synapomorphy, together with the strong molecular evidence that *T. tentaculata* is a member of the *Roboastrea* clade, compel us to transfer *T. tentaculata* to *Roboastrea* to preserve the monophyly of *Roboastrea*.

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